ZOOLOGICAL SCIENCE 11: 583–588 (1994)

Several Biochemical Alterations from Larval to Adult Types are Independent on Morphological Metamorphosis in a Salamander, *Hynobius retardatus*

Masami Wakahara, Norihisa Miyashita, Akane Sakamoto and Takako Arai

Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060, Japan

ABSTRACT—Biochemical transitions from larval to adult types, such as changes in hemoglobin subunits and pattern of excretion of nitrogen wastes, were studied during ontogeny of a salamander, *Hynobius retardatus*, which had been reported to show neotenic reproduction. A transition of hemoglobin subunits in normally metamorphosing and metamorphosed, and T_4-induced precociously metamorphosed *H. retardatus* was analyzed using SDS-PAGE. The transition of hemoglobin subunits from larval to adult types occurred on the same time schedule in both normally metamorphosing and precociously metamorphosed animals. A changeover from ammonotelism to ureotelism was analyzed by determining amounts of ammonia and urea excreted from normal and metamorphosis-arrested animals. A basic changeover from ammonotelism to ureotlism also occurred even in metamorphosis-arrested, aquatic larvae, on the similar time schedule in normally metamorphosing and metamorphosed animals. Because the transition of hemoglobin subunits in the metamorphosis-arrested larvae has been reported to occur on the same time schedule as in the controls, it is concluded that either transitions of hemoglobin subunits from larval to adult or pattern of nitrogen excretion from ammonotelism to ureotelism are independent on the morphological metamorphosis in *H. retardatus*. The substantial separation of biochemical "metamorphosis" from morphological metamorphosis will explain a possible cause of neoteny which has been reported in this species.

INTRODUCTION

Biochemical alterations in metamorphosing amphibian larvae have met with considerable interest as criteria illuminating the evolutionary past of these animnals, or as traits of adaptive significance for the transition from the aquatic to the terrestrial habitat [24]. Among various biochemical alterations, changes in molecular constituents of the body such as blood proteins [6], keratins [18] and hemoglobins subunits [4, 13, 24], and a pattern of nitrogen excretion [6, 17, 24] were extensively studied using many amphibian speceis. For instances, a switch in hemoglobin synthesis is reported to occur at metamorphosis, resulting in the replacement of the larval globin subunits by a set of distinct adult ones [9, 16]. Similarly to the hemoglobin transition, a pattern of nitrogen waste pruducts is known to change during metamorphosis in many species of amphibians which show a shift of habitats from aquatic to terrestrial according to the metamorphosis: excretion of ammonia exceeds that of urea during pre- and prometamorphic stages, whereas the situation is reversed in postmetamorphic and adult stages [24]. The shift from ammonotelism to ureotelism coincides with the metamorphic climax.

Contrary to anurans and ordinary urodelans, something different phenomena are known in neotenic urodelans. Axolotl, a neotenic form of *Ambystoma mexicanum*, has been reported to show the transition of hemoglobins from larval to adult types without any indications of anatomical metamorphosis [5, 13]. Similar has happened in *Hynobius retardatus*, which was reported to show neotenic reproduction in the specific environment of Lake Kuttara, a small volcanous lake not far from Sapporo [19, 20]: the transition of hemoglobin subunits from larval to adult types occurs on the same time schedule in both normally metamorphosing and metamorphosed animals and metamorphosis-arrested larvae [1]. Furthermore, we have recently demonstrated that *H. retardatus* can produce morphologically mature spermatozoa even in larval forms with well developed gills and tail fins when the metamorphosis is arrested by goitrogens [23]. Since *H. retardatus* shows very similar pattern of the transition of hemoglobins to that in the axolotl, and has an ability to undergo neoteny [19, 20], this salamander is expected to have similar biochemical characteristics to the axolotl or other salamanders showing the facultative neoteny. In the present study, a hemoglobin transiton from larval to adult types and a changeover of pattern of the nitrogen excretion from ammonotelism to ureotelism were analyzed using experimentally induced, precociously metamorphosed animals and metamorphsosis-arrested larvae of *H. retardatus*.

Accepted June 17, 1994
Received April 11, 1994

MATERIALS AND METHODS

Animals

Fertilized eggs of *Hynobius retardatus* were collected from several ponds or small streams in the vicinity of Sapporo in the

breeding season. Newly hatched larvae were reared at 10°, either in an aqueous solution of diluted thyroxine (T_4, 6×10^{-8} M) in order to induce a precocious metamorphosis, or in T_4-free solution as controls. Others were reared at a room temperature either in aqueous solution of 0.02% thiourea (TU) plus 0.02% sodium perchlorate (SPC) to arrest the metamorphosis, or goitrogen-free medium as controls. They were fed with live *Tubifex*. After they metamorphosed, they were transferred to a terrarium. Developmental stages were determined according to the normal table for *Hynobius nigrescens* [10].

Identification of hemoglobin subunits

Procedures for preparation of hemolysates from larvae, juveniles and adults were described previously [1]. After the amount of protein was determined using BCA Protein Assay Reagent (Pierce Chem. Co.), the samples were electrophoresed or frozen at −80°C. Samples from very small larvae were combined together because the amount of hemolysate from one larva was too small. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli [11], using 15% separating gels [1]. All electrophoresed gels were stained with Coomassie Brilliant Blue.

Determination of amount of ammonia and urea

Each experimental larva or juvenile was placed in a Petri dish or grass beaker with a cover of proper size filled with 20 ml of 20 mM phosphate buffer (pH 6.8) for 24 hr. After removing unnecesary solids from the solution including excretion of laevae or juveniles with a centrifugation (3000 g, 7 min), a part of the samples was directly used for determination of amounts of ammonia as well as urea. The amount of ammonia was determined by means of phenol-chloramine T-spectrophotometry [8]. The amount of urea was determined according to the procedure by Archibald [2]. Three to 6 larvae or juveniles were used for one determination.

RESULTS

Larval development under various rearing conditions

Fig. 1 shows time courses of development in *H. retardatus* which were reared at 10°C, either in T_4 or T_4-free media. At 10°C which was employed to retard the metamorphosis in controls in order to make conspicuous the effects of T_4, the progress in larval development and metamorphosis was relatively slow. Almost all control larvae developed to stage 63, fully grown larval stage just before metamorphosis [10], 100–110 days after hatching [1]. Contrary to this, the larvae treated with T_4 developed much faster: they reached stage 63 approximately 50 days after hatching.

A wide variation in the progress in larval development and metamorphosis was observed at 10°C: some controls could complete metamorphosis by 180 days, but others could not complete even after 250 days of hatching (cf. [1]). At the end of this experiment (250 days after hatching), the average developmental stage of controls was stage 67.3, almost completion of morphological metamorphosis. The animals which were reared in T_4 completed metamorphosis approximately by 130 days after hatching. This indicated convincingly that exogeneously applied T_4 accelerated the metamorphosis in experimental groups for 60–90 days.

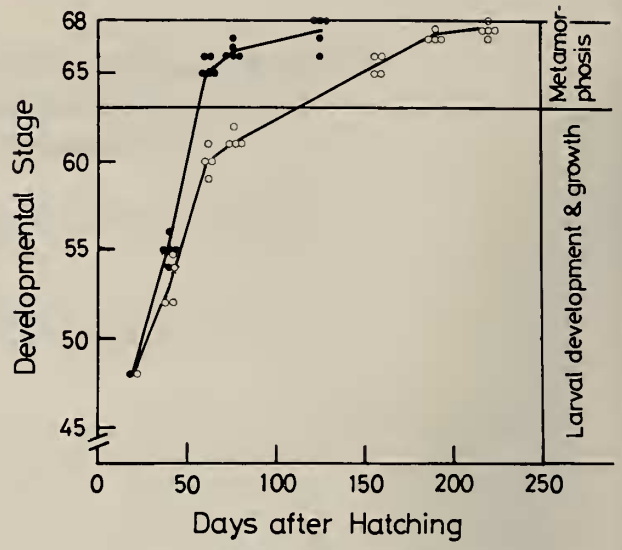

Fig. 1. Progress in larval developmental stages in *Hynobius retardatus*. Larvae just after hatching were reared in thyroxine (T_4) (closed circles) or T_4-free (open circles) media at 10°C. The larval developmental stages were determined according to Iwasawa and Yamashita [10]. The larvae treated with T_4 reach stage 63, fully grown larval stage just before metamorphosis, approximately by 50 days after hatching, whereas almost all controls develop to stage 63 after 100–110 days of hatching. The animals treated with T_4 complete metamorphosis by 130 days after hatching, whereas almost all controls metamorphose 220 days after hatching.

When the larvae were reared in an aqueous solution of thiouea (TU) and sodium perchlorate (SPC) at a room temperature, morphological metamorphosis was basically blocked at stage 64–65. Although all controls metamorphosed by 70 days after hatching, proceeding of the metamorphosis in the goitrogen-treated larvae was extremely retarded or basically arrested (see Fig. 5 of [1]). No larvae treated with goitrogens completed morphological metamorphosis within this experiment (250 days after hatching). All larvae reared in goitrogens had external gills and well developed tail fins which were characteristic to aquatic forms, and appeared to adapt to the water habitat (Fig. 2).

Hemoglobin transition

Fig. 3 shows electrophoretic profiles of hemoglobin subunits from normally metamorphosing and metamorphosed animals reared at 22°C and 10°C. Typical larval globins were separated into 2 bands (L1 and L2) on SDS-PAGE. Approximate molecular weights of them were 13700 and 15000. Adult hemoglobins were shown to be composed of 3 fractions (A1 to A3) whose molecular weights were estimated as 14000, 15000 and 15500, respectively. Adult type subunits were already detected in the larvae of 35 days after hatching at 22°C (Panel A, lane a, arrowheads). Similarly to this, adult type subunits were observed in the larvae of 126 days after hatching at 10°C. Inversely, the larval type bands were getting faint (Panel B, lane a, arrowhead). In the larvae of 189 days (when the average developmental stage

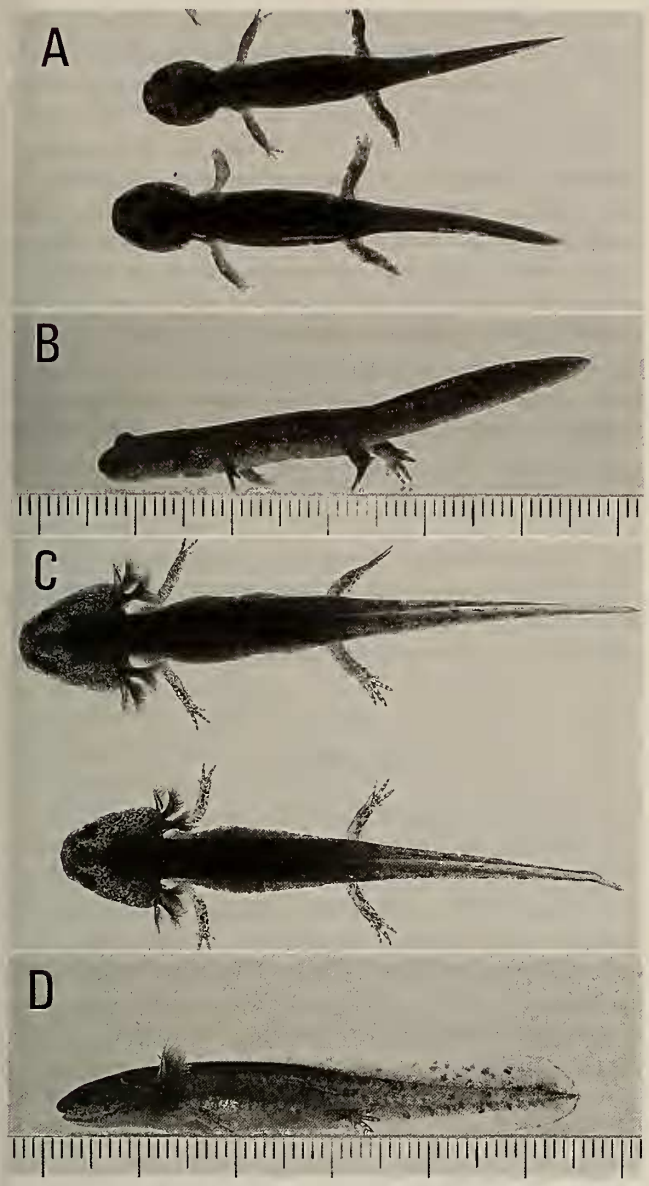

FIG. 2. External morphology of metamorphosed *Hynobius retardatus* and metamorphosis-arrested larvae. Larvae just after hatching were reared in an aqueous solution of thiourea plus sodium perchlorate or in the goitrogen-free (control) media at room temperature. Dorsal (A) and lateral (B) views of metamorphosed controls on 68 days after hatching. Dorsal (C) and lateral (D) views of metamorphosis-arrested larvae of the same age as the metamorphosed controls. All controls complete metamorphosis 70 days after hatching and shift to terrestrial habitats. Metamorphosis-arrested larvae have well developed external gills and tail fins, and fully adapt to the aquatic habitat.

was stage 66.5), the larval bands could not be detected on SDS-PAGE (Panel B, lane c). This indicates that the transition of hemoglobins from larval to adult types finishes before the completion of morphological metamorphosis in the larvae reared at 10°C.

Fig. 4 shows electrophoretic profiles of hemolysates from T_4-treated and T4-free animals reared at 10°C. Adult bands did not detected on 40 days after hatching (lanes a, b, c), but

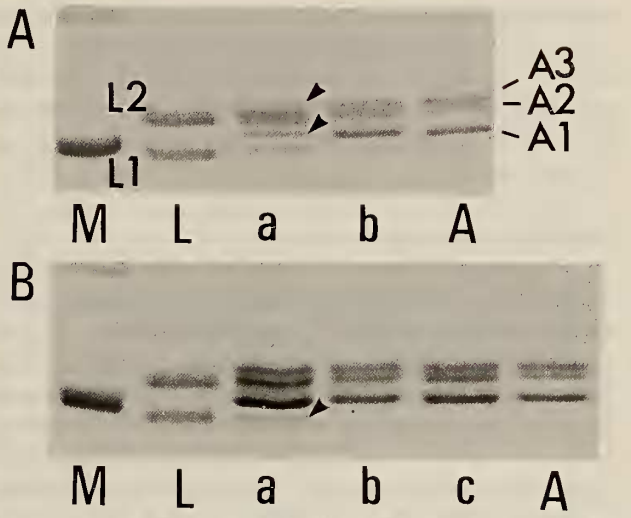

FIG. 3. Typical electrophoregrams of hemoglobins in *Hynobius retardatus*. Time courses in the transition of hemoglobins from larval to adults types at 22°C (A) and 10°C (B) were analyzed on SDS-PAGE. Panel A, lane M, molecular marker (top, 20K, bottom, 14K); L, hemolysate from typical larvae (3 days after hatching); a, 35 days after hatching; b, 68 days after hatching; A, hemolysate from typical adult. Larval hemoglobins are separated into 2 bands (L1 and L2). Adult globins are separated into 3 bands, A1 to A3. Adult type subunits are clearly seen in 35 days larvae (lane a, arrowheads). Panel B, lane M, molecular marker; L, typical larvae; a, 126 days after hatching; b, 159 days after hatching; c, 189 days after hatching; A, typical adult. Larval type subunits are getting faint in 126 days (lane a, arrowhead) and disappeared in 189 days larvae (lane c).

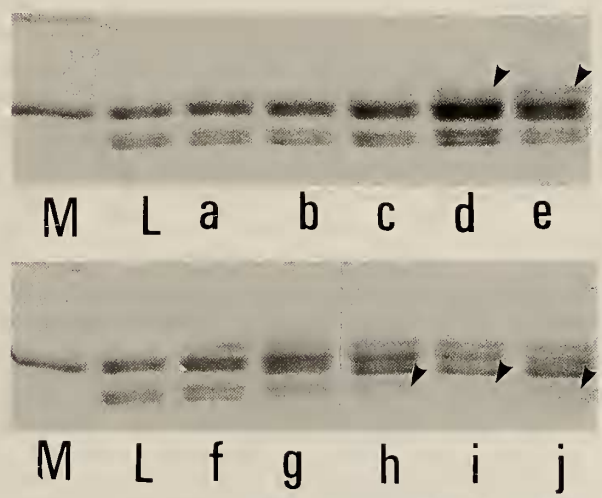

FIG. 4. Electrophoretic profiles on SDS-PAGE showing the transition of hemoglobins from larval to adult types. Hemolysates were prepared from precociously metamorphosed (T_4-treated) and control (T_4-free) animals at 10°C. Lane M, molecular marker (top, 20 K, bottom, 14 K); lane L, typical larval hemolysate; lanes a and b, control, 40 days; lane c, T_4-treated, 40 days; lane d, control, 68 days; lane e, T_4-treated, 68 days; lane f, control, 78 days; lane g, T_4-treated, 78 days; lane h, control, 157 days; lanes i and j, T_4-treated, 157 days. Adult type subunits appear on 68 days after hatching in both T_4-treated, and T_4-free animals (arrowheads on lanes d and e). Larval type subunits in the controls are getting faint on the same time cources as in the precociously metamorphosed animals (arrowheads on lanes h, i and j).

faintly appeared on 63 days after hatching in both T_4-treated, precociously metamorphosing animals (lane e) and T_4-free, normal control (lane d). Similarly to these, disappearance of larval bands in precociously metamorphosed animals began on the same time schedule as in the controls (lanes h, i, j; 157 days after hatching).

Pattern of nitrogen excretion

Amount of nitrogen (N) out of ammonia and urea excreted from larvae and juveniles during the ontogeny in normal controls and in the metamorphsosis-arrested larvae was determined at the level of μg N/body wight (g)/day. Fig. 5 shows combined data from three different experiments demonstrating changeover from ammonotelism to ureotelism during the ontogeny and in the metamorphosis-arrestesd larvae of *H. retardatus*. Each point on the graph indicates an average of 3 to 6 determinations using individual larvae or juveniles, respectively. Although considerable variations were found in each experiment, it was clear a major nitrogen

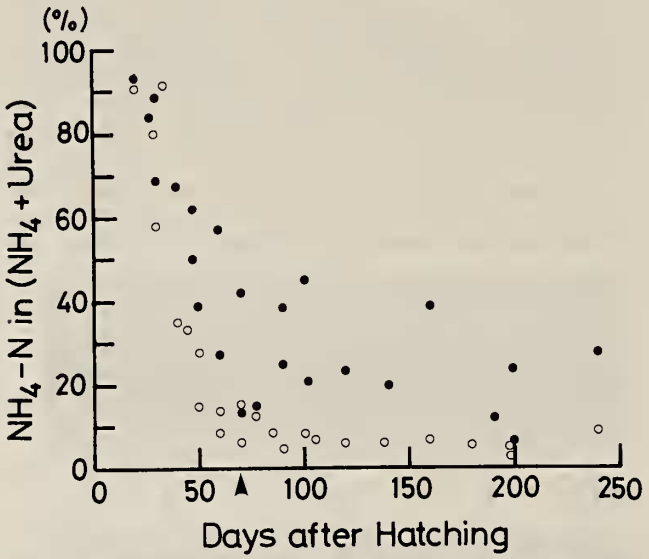

Fig. 5. Changes in the pattern of nitrogen excretion from ammonotelism to ureotelism in *Hynobius retardatus*. Amounts of ammonia and urea excreted from individual animals were measured respectively, and ratio of ammonia-nitrogen (NH_4-N) to total-(NH_4+urea)-N was calculated. An arrowhead on abscissa indicates the completion of metamorphosis in controls. In the controls (open circles), approximately 90% of the (NH_4+urea)-N is ammonia before and during early metamorphosis. After the metamorphosis, the ratio of ammonia-N to the (NH_4+urea)-N is maintained at 5–10% (in average, 7.0±3.3%) level. In the metamorphosis-arrested larvae (closed circles), approximately 80–90% of the (NH_4+urea)-N is ammonia during the early larval stages, similarly to the controls. After the controls completed metamorphosis, the ratio of ammonia-N in the metamorphosis-arrested larvae gradually decreases to the level of 10–40% (in average, 25.0±12.0%). Level of the ratio of ammonia-N to the (NH_4+urea)-N in controls after the metamorphosis is significantly lower than in the metamorphosis-arrested larvae ($p<0.01$ in Student's t test). These are combined data from three different experiments. Each point indicates an average of 3 to 6 determinations using individual larvae or juveniles, respectively.

waste was ammonia in larval stages both in control and metamorphosis-arrested groups. In the controls (Fig. 5, open circles), approximately 90% of nitrogenous wastes (nitrogens of ammonia plus urea) was ammonia before metamorphosis and during early metamorphosis. During metamorphosis, however, the ratio of ammonia-N drastically decreased and then maitained at a 5–10% level after the metamorphosis. In metamorphosis-arrested larvae (Fig. 5, closed circles), the pattern of nitrogen excretion was basically same as in the controls during early development: approximately 80–90% of nitrogenous wastes was ammonia. After the controls completed metamorphosis, the major nitrogen waste products gradually change from ammonia to urea in the metamorphosis-arrested larvae, even though they showed typical larval forms morphologically (Fig. 2). Timing of the increase in urea excretion (conversely the decrease in ammonia excretion) in the metamorphosis-arrested larvae was a little later than that in the controls. Furthermore, the level of ammonia excreted from the controls after metamorphosis (in average, 7.0±3.3% of total nitrogen) was significantly lower than that of the metamorphosis-arrested larvae (25.0± 12.0%).

DISCUSSION

Transition of hemoglobin subunits

Although the beginning and completion of the metamorphosis are accelerated in T_4-treated animals for 50–90 days compared with the normal controls reared at 10°C, the transition of the hemoglobin subunits from larval to adult types occurs on the same time schedule in both control and experimental groups. This suggests convincingly that though exogeneously applied thyroid hormone induces precocious metamorphosis at the morphological level, it does not induce biochemical precocious "metamorphosis". This result supports furthermore our previous results, which demonstrated the hemoglobin transition occurred even in metamorphosis-arrested larvae on the same time schedule as in the controls [1], and is consistent with the observations showing the transition was completely independent on the morphological metamorphosis in axolotl [5, 13].

Because hemoglobins from metamorphosing larvae of *H. retardatus* were separated into 8 different polypeptides on the two-dimentional electrophoresis [1], the globin subunits are considered to be encoded by 8 different genes, 4 larval and 4 adult genes whose expressions are developmentally regulated. In many anurans, it is reported that the larval hemoglobin subunits stop being produced after a thyroid hormone treatment due to the down-regulation of the corresponding genes, whereas the genes coding the adult hemoglobin subunits are up-regulated [25]. Contrary to these, an earlier observation demonstrated that the hemoglobin transition in *Xenopus laevis* was determined more by chronological age, or size, or some other independent factors, rather than the hormonal control by thyroid hormones [14]. The present results which have shown an independence of the

transition of hemoglobin subunits on exogeneously applied T_4 are consistent with the latter: the hemoglobin transiton in *H. retardatus* is possibly determined by chronological age.

Pattern of nitrogen excretion

The conversion of the pattern of nitrogen excretion has been thought to be controlled internally by the effects of thyroid hormones [24] and/or externally by environmental conditions where the animals are placed [3, 12, 15]. Since the transition occurred during the climax of metamorphosis in normal controls (Fig. 5), it is assumed that increasing concentrations of the circulating thyroid hormones play some roles on this transition in *H. retardatus* as well. This explanation is consistent with the fact that the level of the urea excretion was relatively lower in the metamorphosis-arrested larvae than in the controls, probably due to a shortage of thyroid hormones in the former.

Because the goitrogens used in this experiment are considered to suppress substantially the thyroid activity [23], concentrations of the circulating thyroid hormones in the metamorphosis-arrested laevae was expected to be very low so that the morphological metamorphosis was basically arrested [1, 23]. In spite of the fact, a substantial transition from ammonotelism to ureotelism occurred in the metamorphosis-arrested larvae, though the timing of the transition was a little later than the conspicuous transition in the controls (Fig. 5). Because it has been reported that a major nitrogen excretion in the axolotl is urea [21], it seems possible that the metamrophosis-arrested larvae in *H. retardatus* excrete nitorogenous wastes as urea, even though they adapted to the aquatic habitat. A possible explanation for this is as follows: the substantial transition from ammonotelism to ureotelism in the metamorphosis-arrested larvae is regulated by thyroid hormones of very low concentrations which are insufficient to induce morphological metamorphosis. This will explain a retardation of the transition, and an insufficient transition in the metamorphosis-arrested larvae compared with the controls (Fig. 5).

The detoxication of ammonia constitutes an important biochemical adaptation to the restriction of water supply in amphibians which shift from aquatic to terrestrial habitats [3]. Thus, *Xenopus laevis*, which lives in aquatic forms even after metamorphosis, excretes major nitrogen waste products as ammonia [17, 24]. Furthermore, changes in the pattern of nitrogen excretion from ammonotelism to ureotelism have been reported after adult *Xenopus* has been exposed to restricting water supply [3], or high osmolarity [15]. From this point of view, aquatic larvae of *H. retardatus* which have been treated with the goitrogens and axolotl need not to change the pattern of nitrogen excretion from ammonotelism to ureotelism, because they are surrounded by a lot of water and fully adapted to an aquatic habitat. The fact that the ratio of ammonia-nitrogen excreted from metamorphosis-arrested larvae (10–40%, in average $25.0 \pm 2.0\%$) was considerably higher than that from controls (5–10%, in average $7.0 \pm 3.3\%$) will answer to this: although a substantial transiton is expected to be induced by thyroid hormones of very low concentrations, an aquatic environment resulting from the incompletion of the metamorphosis affects on the mechanism of nitrogen excretion, probably on the activity of enzymes of the ornithine cycle in liver [12]. Thus, the alteration of nitrogen excretion in *H. retardatus* will be regulated by a combination of hormonal controls and environmental conditions. Since it was difficult to treat separately hormonal (internal) and environmental (external) conditions, degrees of their involvement in the transiton of nitrogen excretion were not examined in this study. Determinations of the concentrations of circulating thyroid hormones are necessary to elucidate these, and now in progress.

Heterochrony in phenotypic expression

Although the transition of hemoglobin subunits from larval to adult types occurs on the same time schedule in both normally metamorphosing and metamorphosis-arrested animals [1], the transition of nitrogen excretion from ammonotelism to ureotelism in the metamorphosis-arrested larvae occurs a little later than in the controls. Furthermore, it has been reported that the gonadal development is much earlier than the somatic development in metamorphosis-arrested *Hynobius* [23]. These chronological differences in phenotypic expression or development imply possible differences in the sensitivity to thyroid hormones among various tissues. Since axolotls are reported to be able to produce thyroid hormones at a very low level, and accomplish a number of cryptic metamorphic processes [22], different tissues or cells will behave differently in response to the thyroid hormones. Thus, it is possible that the morphological or anatomical metamorphosis such as disappearance of external gills and tail fins is accelerated by relatively high concentrations of circulating thyroid hormones, but that the biochemical "metamorphosis" such as the transition of nitrogen excretion is regulated by very low concentrations of thyroid hormones and/or in part by environmental conditions. The transition of hemoglobin subunits and the developement of germ cells will be regulated by some other factors, rather than the hormonal control by thyroxine. These heterochronic phenotypic expressions or development will be fundamental causes of the reported neoteny in this species [7].

REFERENCES

1 Arai T, Wakahara M (1993) Hemoglobin transition from larval to adult types in normally metamorphosing, metamorphosed and metamorphosis-arrested *Hynobius retardatus*. Zool Sci 10: 637–644

2 Archibald RM (1945) Colorimetric determination of urea. J Biol Chem 157: 507–518

3 Balinski JB (1961) Adaptation of nitrogen metabolism to hyperosmotic environment in Amphibia. J Exp Zool 215: 335–350

4 Cardellini P, Sala M (1979) Metamorphic variations in the hemoglobins of *Bombina variegata* (L.). Comp Biochem Physiol 64B: 113–116

5 Ducibella T (1974) The occurrence of biochemical metamorphic events without anatomical metamorphosis in the axolotl. Devel Biol 38: 175–186

6 Frieden E (1961) Biochemical adaptation and anuran metamorphosis. Am Zool 1: 115–149

7 Gould SJ (1977) "Ontogeny and Phylogeny". Harvard University Press, Cambridge

8 Grismer MR (1937) Colorimetric determination of ammonia. Bull Soc Chim Biol 19: 1000–1006

9 Hosbach HA, Widmer HJ, Andreas A-C, Weber R (1982) Expression and organization of the globin genes in *Xenopus laevis*. In "Embryonic Development, Part A: Genetic Aspects". Ed by MM Burger, Weber R, Alan R Liss, New York, pp 115–125

10 Iwasawa H, Yamashita K (1991) Normal stages of development of a hynobiid salamander, *Hynobius nigrescens* Stejneger. Jpn J Herpetol 14: 39–62 (in Japanese with English Abstract)

11 Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685

12 Lee AR, Silove M, Katz U, Balinski JB (1982) Urea cycle enzymes and glutamate dehydrogenase in *Xenopus laevis* and *Bufo viridis* adapted to high salinity. J Exp Zool 221: 169–172

13 MacLean N, Jurd RD (1972) The control of haemoglobin synthesis. Biol Rev 47:393–437.

14 MacLean N, Turner S (1976) Adult hemoglobin in developmentally retarded tadpoles of *Xenopus laevis*. J Embryol Exp Morph 35:261–266

15 McBean RL, Goldstein L (1970) Accelerated synthesis of urea in *Xenopus* during osmotic stress. Am J Physiol 219: 1124–1130

16 Moss B, Ingram VM (1968) Hemoglobin synthesis during amphibian metamorphosis II. Synthesis of adult hemoglobin following thyroxine administration. J Mol Biol 32: 481–504

17 Munro AF (1953) The ammonia and urea excretion of different species of Amphibia during their development and metamorphosis. Biochem J 54: 29–36

18 Nishikawa A, S-Nishikawa K, Miller L (1992) Spatial, temporal, and hormonal regulation of epidermal keratin expression during development of the frog, *Xenopus laevis*. Devel Biol 151: 145–153

19 Sasaki M (1924) On a Japanese salamander, in Lake Kuttarush, which propagates like the axolotl. J Coll Agr Hokkaido Imp Univ 15: 1–36

20 Sasaki M, Nakamura, H (1937) Relation of endocrine system to neoteny and skin pigmentation in a salamander, *Hynobius lichenatus* Boulenger. Annot Zool Japon 16: 81–97

21 Schultheiss H (1977) The hormonal regulation of urea excretion in the Mexican axolotl (*Ambystoma mexicanum* Cope). Gen Comp Endocrinol 31: 45–52

22 Tompkins R (1978) Genetic control of axolotl metamorphosis. Am Zool 18: 313–319

23 Wakahara M (1994) Spermatogenesis is extraordinarily accelerated in metamorphosis-arrested larvae of a salamander, *Hynobius retardatus*. Experientia 50: 94–98.

24 Weber R (1967) Biochemistry of amphibian metamorphosis. in "The Biochemistry of Animal Development, vol 2". Ed by R Weber Academic Press, New York, pp 227–310

25 Widmer BA, Andreas AC, Niessing J, Hosbach HA, Weber R (1981) Comparative analysis of cloned larval and adult globin cDNA sequences of *Xenopus laevis*. Devel Biol 88:325–332